

Preliminary analysis of Stearoyl Co-A Desaturase gene transcripts in River buffalo

A. Pauciullo, G. Cosenza, A. D'Avino, L. Colimoro, C. Iorio,
D. Nicodemo, D. Di Berardino, L. Ramunno

Department of Soil, Plant, Environment and Animal Production Sciences;
Faculty of Agriculture, University of Naples "Federico II", Portici (Na), Italy

Corresponding author: L. Ramunno. Department of Soil, Plant, Environment and Animal Production Sciences; University of Naples "Federico II"; Via Università 133, 80055 Portici (Na), Italy - Tel. +39/0812539004 - Fax: +39/0817762886 - Email: ramunno@unina.it

ABSTRACT: Stearoyl-CoA desaturase (SCD) is a key enzyme in the biosynthesis of monounsaturated fatty acids (MUFAs). In cattle, SCD gene extends over a DNA segment of ~17.0 Kb, and it is organized in 6 exons and 5 introns. The SCD gene has been indicated as the candidate gene to change the saturated/unsaturated FAs ratio and hence it has been suggested as the gene influencing the fat quality. In cattle, eight SNPs have been identified and one of them, (T→C) at 231st nt of 5th exon, is responsible for the Val→Ala amino acid change. The C allele has been associated with higher content of MUFAs in carcasses, and it is positively related to a higher index of desaturation (C18:0/C18:1 and C16:0/C16:1) in the milk. In this study, we report on preliminary results of analysis of transcripts of the SCD encoding gene in river buffalo. The electrophoretic analysis of the RT-PCR products and the subsequent sequencing showed at least five different populations of mRNA. The most represented population is correctly assembled (~1300 bp), followed by the one which is deleted of ~750bp, corresponding to the 3rd, 4th and 5th exon and partially to the 2nd and 6th exon.

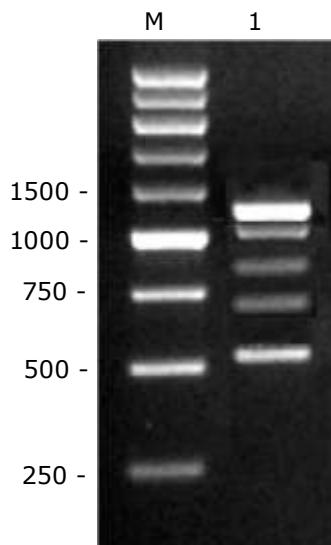
Key words: River buffalo, Stearoyl Co-A Desaturase, SCD gene, Transcripts.

INTRODUCTION - Stearoyl-CoA desaturase is a key enzyme in the biosynthesis of monounsaturated fatty acids (MUFAs) and it plays a central role in regulation of FA metabolism (Heinemann and Ozols, 2003), catalyzing the insertion of a double bond between carbon atoms 9 and 10 in a spectrum of saturated fatty acids (Palmquist *et al.*, 1993). SCD is responsible for saturated/monounsaturated FAs ratio in the composition of the triacylglycerol and in the phospholipids membrane. The alteration of this ratio has been implicated in many diseases, such as diabetes, obesity and cardiovascular diseases (Heinemann and Ozols, 2003). In cattle, the SCD gene extends over a DNA segment of ~17.0 Kb and it is organized in 6 exons and 5 introns. The complete bovine SCD mRNA spans 5.3 Kb and it codes for a protein of 359 amino acids. The SCD gene has been indicated as the candidate gene to change the saturated/unsaturated FAs ratio and therefore, to influence the fat quality (Taniguchi *et al.*, 2004). So far, eight SNPs have been identified in cattle, forming two different haplotypes: A and B. Three SNPs are located on the 5th exon and one of them, (T→C) in position 231st, is responsible for the Val→Ala amino acid change (Taniguchi *et al.*, 2004). The C allele has been associated with higher content of MUFAs in carcasses, and it

is positively related to a higher index of desaturation measured by the ratios (C18:0/C18:1 and C16:0/C16:1) in the milk (Medrano, 2002). In river buffalo very little information on SCD gene is available. Nowadays, only the sequences of the 4th, 5th and 6th exon of the SCD gene are known (DQ088625; DQ646700; DQ646701). In the present study we report on a preliminary characterization of the river buffalo SCD gene transcripts.

MATERIAL AND METHODS - Total RNA was extracted from milk somatic cells of six lactating Mediterranean river buffaloes of comparable age, type of feed, diet, feeding level and lactation stage. Primers for amplification and sequencing were designed by DNA-sis software (Hitachi) using as template the sequence of the bovine *SCD* gene (EMBL: AY241932). The reverse transcription of total RNA was performed using an oligo dT(18). RT mix (20- μ l) was performed using Improm-II Reverse Transcriptase (Promega). PCR was carried out using the following primers: *forward* 5'-CAGCGGAAGGTCCCGA-3' and *reverse* 5'-TGGGTTAACGTCATCTTTAGCA-3', corresponding respectively to nt 3-18 of the 1st exon and complementary to nt 290-311 of the 6th exon of the bovine *SCD* gene. The 100- μ l PCR reaction mix comprised: the 20- μ l of RT product, 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% Triton X-100, 2 mM MgCl₂, 10 pmol of each primer, 0.2 mM each dNTPs, 5 U of Taq DNA Polymerase (Promega). Amplification consisted of initial denaturation at 95°C for 4 min followed by 37 cycles carried out at 95°C for 45 sec, 62°C for 45 sec, 72°C for 90 sec, and 10 min of final extension in the last cycle. All the amplicons were analysed by electrophoresis on 1.5 % agarose gel in TBE 1X buffer. Densitometric analysis of RT-PCR products was displayed using QuantityOne software (BioRad). PCR products were purified from gel with QIAquick kit (QIAGEN) and sequenced, twice in both directions, according to Sanger *et al.* (1977) method.

Figure 1. RT-PCR (1st – 6th exon) of mRNA transcribed from river buffalo SCD gene. M: Marker 1000 bp (Promega); lane 1: RT-PCR product.



RESULTS AND CONCLUSIONS - The electrophoresis analysis of the RT-PCR products of six lactating Mediterranean river buffaloes showed patterns characterized by at least five bands of sizes ~1300 bp, ~1100 bp, ~900 bp, ~700 bp and ~550 bp (Figure 1).

The most represented mRNA population is ~1300 bp long (~52%), followed by the one of about 550 bp (~30%). These two RT-PCR products have been sequenced. The first sequenced transcript is 1250 bp long (EMBL acc. no. AM600640) and it shares a total similarity with the corresponding bovine cDNA of ~97.8%. The subsequent comparison with the bovine *SCD* gene allowed the subdivision in 6 exons (from the 53rd nt of the exon 1 to the 278th nt of the exon 6), confirming that such transcript is correctly assembled and it

Figure 2. Homology between the river buffalo SCD cDNA sequences of the correctly assembled transcript (upper line) and the incorrectly assembled. Dashes represent missing nucleotides, capital and small letters are translated and untranslated exons respectively, bold letters are amino acids.

	10	20	30	40	50	60	70	80	90	
1	tcaggaacta	gtctacactc	agtttggact	gccccgaact	cogctcogca	gtctcagccc	cgagaaagtg	atcccagtg	cctagaggcc	90
1	tcaggaacta	gtctacactc	agtttggact	gccccgaact	cogctcogca	gtctcagccc	cgagaaagtg	atcccagtg	cctagaggcc	90
	100	110	120	130	140	150	160	170	180	
	M P A N H L L Q E E I S S S Y T T T T T I T A P P S R V L Q									
91	agATGCCGGC	CCACTTGGTG	CAAGAGGAGA	TCTCTAGCTC	CTACACAACC	ACCACCACCA	TCACACACCC	TCCTTCCAGG	GTCTCGCAGA	180
91	agATGCCGGC	CCACTTGGTG	CAAGAGGAGA	TCTCTAGCTC	CTACACAACC	ACCACCACCA	TCACACACCC	TCCTTCCAGG	GTCTCGCAGA	180
	190	200	210	220	230	240	250	260	270	
	N G G G K L E K T P L Y L E E D I R P E T R D D I Y D P T Y									
141	ATGAGAGGGG	CAAAITGGAA	AAGACTCCCC	TATACTTGGG	AGAAGACATC	CGCCCTGAAA	CGAGAGATGA	CATCTATGAC	CCAACCTACC	270
141	ATGAGAGGGG	CAAAITGGAA	AAGACTCCCC	TATACTTGGG	AGAAGACATC	CGCCCTGAAA	CGAGAGATGA	CATCTATGAC	CCAACCTACC	270
	280	290	300	310	320	330	340	350	360	
	Q D K E G P K F P K L E Y V W R N I I L M F L L H L G A L Y G									
271	AGGATAAGGA	GGGCCCAAG	CCCAAGCTTG	AGTATGTTTG	GAGAAACATC	ATCCTCATGT	TTCTGTATCA	CTTGGGAGCC	CTATATGGGA	360
271	AGGATAAGGA	GGGCCCAAG	CCCAAGCTTG	AGTATGTTTG	GAGAAACATC	ATCCTCATGT	TTCTGTATCA	CTTGGGAGCC	CTATATGGGA	360
	370	380	390	400	410	420	430	440	450	
	I T L I P T C K I Y T Y L W V L F Y Y L M G A L G I T A G A									
361	TCATATGAT	CCCCACTGCG	AAGATATAGA	CTCTACTCTG	GGTGTATATC	TACTATCTGA	TGGGTGCCCT	GGGCATGACA	GCAGGGGGCC	450
361	TCATATGAT	CCCCACTGCG	AAGATATAGA	CTCTACTCTG	GGTGTATATC	TACTATCTGA	TGGGTGCCCT	GGGCATGACA	GCAGGGGGCC	450
	460	470	480	490	500	510	520	530	540	
	H R L W S H R T Y K A R L P L R V F L I I G N T M A F Q N D									
451	ATCGCGCTGG	GAGTCACCGA	ACCTACAAAG	CTCGCTGCC	CTCGCGGGTC	TTCTGTATCA	TCGCGACACG	CATGGCGTTC	CAGAATGACG	540
451	ATCGCGCTGG	GAGTCACCGA	ACCTACAAAG	CTCGCTGCC	CTCGCGGGTC	TTCTGTATCA	TCGCGACACG	CATGGCGTTC	CAGAATGACG	540
	550	560	570	580	590	600	610	620	630	
	V F E W S R D H R A H H K F S E T D A D P H N S R R G F F F									
541	TTTTTGAATG	GTCCCGAGAT	CACCGTGCC	ACCACAAGTT	TTCAAGAACG	GATGCTGATC	CCACACAATC	CCGACGTGGC	TTTTTCTTCT	630
541	TTTTTGAATG	GTCCCGAGAT	CACCGTGCC	ACCACAAGTT	TTCAAGAACG	GATGCTGATC	CCACACAATC	CCGACGTGGC	TTTTTCTTCT	630
	640	650	660	670	680	690	700	710	720	
	S H V G W L L V R K H P A V I E K G S T L N L S D L K A E K									
631	CTCAGCTGGG	TTGGCTGCTT	GTCCCGCAAC	ACCCAGCTGT	CATAGAAAG	GGTCTACGC	TAAATTTATC	CGACCTAAAA	CCCGAGAGGC	720
631	CTCAGCTGGG	TTGGCTGCTT	GTCCCGCAAC	ACCCAGCTGT	CATAGAAAG	GGTCTACGC	TAAATTTATC	CGACCTAAAA	CCCGAGAGGC	720
	730	740	750	760	770	780	790	800	810	
	L V M F Q R R Y Y K F G V L L L C F I L P T L V P W Y L M G									
721	TGGTAATGTT	CCAGAGGAGG	TACTACAAAC	CTGGTGTCTT	GTGTTGTGTC	TTCACTCTGC	CCACACTCGT	GCCGTGGTAT	CTGTGGGGTG	810
721	TGGTAATGTT	CCAGAGGAGG	TACTACAAAC	CTGGTGTCTT	GTGTTGTGTC	TTCACTCTGC	CCACACTCGT	GCCGTGGTAT	CTGTGGGGTG	810
	820	830	840	850	860	870	880	890	900	
	E T F Q N S L F F A T L F R Y A L G L N I T W L V N S A A N									
771	AAACGTTTCA	AAACGCTGTC	TTTITTGCCA	CTTATTCCG	TTATGCCCTT	GGGCTCAACA	TCACCTGGCT	GCTGAATAGT	GCTGCCCATATA	900
771	AAACGTTTCA	AAACGCTGTC	TTTITTGCCA	CTTATTCCG	TTATGCCCTT	GGGCTCAACA	TCACCTGGCT	GCTGAATAGT	GCTGCCCATATA	900
	910	920	930	940	950	960	970	980	990	
	M Y G Y R P Y D K T I S P R E N I L V S L G A V G E G F H N									
901	TGTATGATA	TCGCCCTTAT	GACAAGACCA	TCAGCCCCCG	AGAGAAATATT	CTGGTTTCAC	TGGGAGCTGT	GGGTGAGGCC	TTCCACAACCT	990
901	TGTATGATA	TCGCCCTTAT	GACAAGACCA	TCAGCCCCCG	AGAGAAATATT	CTGGTTTCAC	TGGGAGCTGT	GGGTGAGGCC	TTCCACAACCT	990
	1000	1010	1020	1030	1040	1050	1060	1070	1080	
	Y H N T F P Y D Y S A S E Y R W H I N F T T F F I D C M A A									
991	ACCACACAC	CTTTCCTTAT	GACTACTCAG	CCAGTGAGTA	CCGCTGGCAC	ATCAAACTTTA	CCACGTTCTT	CATTGATTGC	ATGGCTGCCA	1080
991	ACCACACAC	CTTTCCTTAT	GACTACTCAG	CCAGTGAGTA	CCGCTGGCAC	ATCAAACTTTA	CCACGTTCTT	CATTGATTGC	ATGGCTGCCA	1080
	1090	1100	1110	1120	1130	1140	1150	1160	1170	
	I G L A Y D R K K V S K A A I L A R M K R T G E S Y K S G *									
1081	TCGGTCTGCG	TTATGACCGG	AAGAAAGTAT	CCAAGGCTGC	CATCTTGGCC	AGGAATGAAA	GAACCTGAGA	GGAAAGCTAC	AAGAGTGCCY	1120
1081	TCGGTCTGCG	TTATGACCGG	AAGAAAGTAT	CCAAGGCTGC	CATCTTGGCC	AGGAATGAAA	GAACCTGAGA	GGAAAGCTAC	AAGAGTGCCY	1120
	1180	1190	1200	1210	1220	1230	1240	1250		
	**									
1121	GAatttggg	tcctttgggt	tccttttcca	aaagccatct	gggcagaggt	ttasatgttct	gtttattaac	tactgaataa	
1121	-----	-----	tccttttcca	aaagccatct	gggcagaggt	ttasatgttct	gtttattaac	tactgaataa	
			S F S K S H L G R G L M F C L L T T E ***							

codifies for 359 amino acids of the protein. The translation stop codon TGA is realized between 198-200 nucleotides of exon 6. A comparison of the sequenced cDNA with the partial published sequences of the buffalo SCD gene (DQ088625; DQ646700; DQ646701) showed 3 polymorphic sites, one of which, realized at the 231st nucleotide of exon 5, is responsible for an amino acid change (GCG^{Ala}→GTG^{Val}). Similar to what has already been observed in cattle (Taniguchi *et al.*, 2004; Medrano, 2002), such transition could be associated to a different content of MUFAs in buffalo carcasses and milk. The remaining two mutations are conservative transversions realized in position 220 of exon 5 (TCC^{Ser}→TCA) and 107

of exon 6 (GCA^{Ala}→GCC), respectively. The second sequenced fragment is 565 bp long and it is a uncorrectly assembled transcript. In fact, the amplicon is deleted of 765 bp, from the 228th nucleotide of exon 2 to the 217th nucleotide of exon 6, probably as a result of an alternative splicing. As a consequence, the corresponding protein is not 359 amino acids long, but probably 104. In fact, as shown in figure 2, the transcript uncorrectly assembled is characterized by the lack of the canonical stop codon (TGA), and the identification of a new stop codon (TAA) realized between the 275-277 nucleotides of the exon 6. Such event would lead to the translation of a part of the 6th exon (last 19 amino acids), normally only transcribed. These findings probably suggest that the skipping of a great part of the SCD exons brings up the accumulation of shortened but stable spliced mRNAs, which enable to encode for a functional protein, but this needs to be further investigated. The preliminary analysis of SCD gene transcripts further increases the interest towards the study of the fatty acid composition in the milk and its elaborated systems of gene regulation.

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